J Appl Electrochem (2014) 44:881–892
DOI 10.1007/s10800-014-0702-2

RESEARCH ARTICLE

Scale-up of manufacturing of printed enzyme electrodes for enzymatic power source applications

Saara Tuurala · Otto-Ville Kaukoniemi · Leo von Hertzen · Johanna Uotila · Anu Vaari · Mikael Bergelin · Pia Sjöberg · Jan-Erik Eriksson · Maria Smolander

Received: 31 March 2014 / Accepted: 9 June 2014 / Published online: 20 June 2014

Abstract Production of printable enzymatic power sources was scaled up from laboratory to roll-to-roll (R2R) pilot production. The anode and cathode enzymes were glucose oxidase (GOx) and laccase, respectively. The best laboratory-scale cells had a maximum power and energy density of 1.4 ± 0.1 μW cm^{-2} and 5.5 ± 0.2 μWh cm^{-2}, respectively. These values are 5 and 28 times higher compared to our previously published values. The R2R-produced cells had a maximum power and energy density of 0.40 ± 0.03 μW cm^{-2} and 0.6 ± 0.1 μWh cm^{-2}, respectively. This is 11 % of the best laboratory manufactured cells. It is suspected that the decrease in electrochemical performance originates from the lower mediator amount and higher drying temperature than that of the laboratory produced cells. However, the trials conducted in this work showed that printed enzymatic active layers can be fabricated and dried with a rotary screen-printing machine in R2R process. Hence, fully printed GOx//laccase power sources could be produced from R2R on a large scale for printed electronics applications.

Keywords Paper-based biofuel cell · Enzymatic power source · Printing · Mass production · Biopower source

Electronic supplementary material The online version of this article (doi:10.1007/s10800-014-0702-2) contains supplementary material, which is available to authorized users.

S. Tuurala (✉) · O.-V. Kaukoniemi · L. von Hertzen · J. Uotila · A. Vaari · M. Smolander
Technical Research Centre of Finland VTT, Tietotie 3, 02150 Espoo, Finland
e-mail: saara.tuurala@vtt.fi

M. Bergelin · P. Sjöberg · J.-E. Eriksson
Åbo Akademi, Biskopsgatan 8, 20500 Åbo, Finland

1 Introduction

The market for printed and thin film electronics is growing fast. By 2021 it was estimated to be worth $44.25 billion, with 56 % on printed and 43 % on flexible substrates [1]. Printed and flexible sensors are playing an increasingly important role in printed electronics, and they already represent a value of $6.3 billion in 2013 [2]. Although currently the biggest market is in disposable glucose biosensors, other types of printed sensors (e.g. temperature and gas sensors) have also entered the market. Due to the growing market for printed electronics, printed power sources are also of interest because they have potentially low manufacturing costs and designs that are easily tailored for different applications.

Environmentally friendly and inexpensive enzyme-based batteries are potential power sources, especially for disposable, low power electronics. Research related to printable enzyme electrodes started at VTT from enzymatic cathodes based on a high redox potential fungal laccase from *Trametes hirsuta* (ThL) by Smolander et al. [3] and Tuurala et al. [4]. A study of immobilization and stabilization of printing-compatible enzymatic anodes based on aldose dehydrogenase (ALDH) was conducted by Tuurala et al. [5]. Construction of fully enzymatic printed biofuel cells based on ThL cathode and glucose oxidase (GOx) and/or ALDH anodes has been studied by Jenkins et al. [6, 7]. All the previous work has been done on laboratory-scale. Hence, in order to prove the capability to mass produce enzyme-based power sources, their printing production needed to be taken from laboratory-scale to pilot-scale.

Printing is one mass-production technique described as the process of transferring ink onto a substrate via a printing plate. In screen-printing process it is possible to

apply a very thick layer of ink (normal values are around 20–100 μm) and to use inks of varying compositions and properties [8]. These two characteristics are the main reasons why screen-printing is suitable for producing enzymatic electrodes. When rotary screen-printing, in which the printing mesh is curved around a cylinder, is used in roll-to-roll (R2R) process one can print continuously on a roll of flexible material ending up with a roll of printed products.

Several parameters have to be considered when aiming for good print quality. The rheology and surface chemistry of the ink are fundamental material characteristics that describe ink flow and surface wetting [9]. Viscosity is the most important rheological characteristic of all liquids, and it has to be at certain level in order for the printing process to proceed in a controllable manner. Too low a viscosity causes ink leakage through the open screen before the actual moment of printing, leading to spread over intended printed patterns. Too high a viscosity causes uneven prints and lost "dots", when ink sticks to the screen and does not flow onto the substrate. For this reason, the rheological behavior of ink should preferably be thixotropic: the ink is thick when it is "resting" and gets thinner when the squeegee presses it through the screen. The adhesion and surface chemistry can be simplified into the strength of attraction between substrate material and ink. The higher the substrate's surface energy relative to the ink's surface tension the greater the attraction.

In addition to the rheology of the ink and surface chemistry between the substrate and ink, drying is a crucial step in R2R manufacturing process. Excessive drying is often used in order to ensure a dry printed surface, which can be rolled onto the winding unit without ink transfer from one printed layer to another. Because the enzymatic activity of inks can be destroyed during excessive drying, appropriate drying conditions for enzyme-containing inks had to be studied before up-scaling the printing process.

The purpose of this work was to investigate how production of printable enzymatic power sources can be scaled up from a laboratory-scale to a R2R pilot production scale. The first trials were conducted in laboratory environment. Two goals were set; (1) to optimize the ink composition to enable R2R processing and (2) to retain the bioelectrochemical performance of the enzymes (Step 1, Fig. 1). Two different water-soluble biocompatible polymers were tested as binders for the enzymatic inks, and the printed layers were characterized electrochemically. In addition, drying of these inks was studied at different temperatures and how the drying affected the enzymatic activity of the printed electrodes (Step 2, Fig. 1). In the last step (Step 3, Fig. 1) of this work, manufacturing of enzymatic electrodes was up-scaled to pilot-scale, which to our knowledge is the first time this has been done. Enzymatically active printed anode and cathode layers were produced and used fully printed enzymatic power sources were assembled. The electrochemical performance of these cells was compared with handmade cells produced in the laboratory.

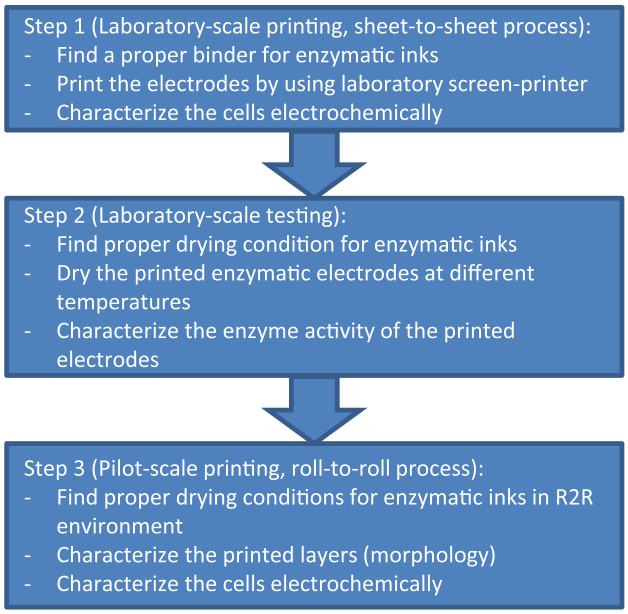

Fig. 1 Illustration of work steps conducted in this work

2 Experimental

2.1 Materials

All chemicals were used as received, and distilled water was used in all water-based solutions. The enzymatic ink constituents were graphite powder (<20 μm, Aldrich 282863), polyethylene oxide (PEO, Aldrich 189456), carboxymethyl cellulose (CMC, Finnfix 10 000 CP Kelco A Huber Company), glucose oxidase from *Aspergillus niger* (GOx, G7141 Sigma), laccase from *Trametes hirsuta* (ThL, VTT), Ecostone laccase (EcoL, Ecostone LcL 45 ABEnzymes), ferrocenemethanol (FeMeOH, 335061 Aldrich), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, A1888 Sigma) and sodium succinic acid (S7501 Sigma-Aldrich). ThL was produced at VTT as described previously by Frasconi et al. [10] using method described by Rittstieg et al. [11].

Other chemicals and materials that were used in this work were ferrocenecarboxaldehyde (122459 Aldrich), ferrocenecarboxylic acid (106887 Aldrich), D-glucose (VWR 101176 K), palladium foil (Alfa Aesar 11517), dialysis membrane (visking code DTV12000.11.000, cutoff 12–14 kDa, Medicell International Ltd.), capacitor paper (Terkab Ilam 33 μm Delfort Group), current collector ink (HAL SD-2843 Peters). Printing substrates that were used

Table 1 Compositions of enzymatic inks for laboratory scale trials

	Anode ink	Cathode ink
Graphite	3 g	3 g
Binder	4.2 g	4.2 g
Enzyme	3000 nkat GOx	3000 nkat laccase (ThL)
Mediator	41 mg FeMeOH	107 mg ABTS
Buffer	app. 1 ml	app. 1 ml

Table 2 Compositions of enzymatic inks for pilot scale trial

	Anode	Cathode
Graphite	25 g	25 g
Binder	18 g	18 g
Enzyme	40,500 nkat GOx	36,700 nkat laccase (EcoL)
Mediator	86 mg FeMeOH	217 mg ABTS
Buffer	10–12 ml	10–12 ml

in this work were electrical insulator paper (Tersep KX 63 μm, Delfort Group), polyimide (Kapton® HN 125 μm, DuPont) and cardboard with a polyethylene (PE) coating (Classic Bar PE 175 + 15, Stora Enso).

2.2 Mediator selection for enzymatic electrodes

ABTS was selected as the mediator for laccase electrodes based on previous studies by Smolander et al. [3] and Jenkins et al. [7]. Previously, two osmium complexes and TMPD have been tested for printed GOx electrodes by Jenkins et al. [6] but the power production was very modest. As a consequence, in this study three ferrocene-based derivatives (ferrocenecarboxaldehyde, ferrocene-methanol and ferrocenecarboxylic acid) were tested as alternative mediators for GOx. CV scans (from −300 to 600 mV vs. Ag/AgCl at 10 mV s^{-1}) of the optional mediators were performed using a glassy carbon working electrode and Ag/AgCl reference electrode in a 50 mM phosphate/citrate buffer (pH 4.5).

In addition, the compatibility of the mediators with GOx from a current output viewpoint was tested by immobilizing GOx into a thin PEDOT film deposited onto a glassy carbon electrode with the method described by Wang et al. [12]. This electrode was immersed into a D-glucose and test-mediator containing electrolyte (0.5 mM), and a CV measurement (from 0 to 400 mV vs. Ag/AgCl at 10 mV s^{-1}) was performed. The solution was constantly stirred to prevent glucose depletion at the active sites of the enzymes during testing.

2.3 Preparation of printed electrodes

2.3.1 Laboratory-scale preparation of enzyme electrodes

Laboratory-scale printing of the enzymatic electrodes was carried out with a semi-automatic Kent SP-400 screen-printer. The printing screen mesh was NMC EX 31-100, and the substrate was insulator paper. Both PEO (5 w% in H_2O) and CMC (1.5 w% in 50 mM Na-succinate buffer) were tested as binders in graphite-based enzymatic inks. The formulation of the inks is shown in Table 1. The inks were screen-printed onto the substrate and dried at room temperature (RT) overnight. The geometrical area of each electrode was 12.25 cm^2.

2.3.2 Pilot-scale preparation of enzyme electrodes

Pilot-scale printing trials were made with rotary screen-printing in VTT's modular ROKO printing machine. Gallus BY Mesh 64 (thickness 200 μm) printing screens were used for all printed layers (see layouts in Online Resource 1). The geometrical area of each electrode was 9 cm^2. The layers were cured on the printing line with three 0.9 m long hot air blasting dryers, and the printing speed was 2 m/min, hence the drying time was 81 s.

Base ink was prepared by mixing together graphite powder and PEO (5 w% in H_2O). The anode ink was prepared by mixing GOx and FeMeOH, and the cathode ink by mixing laccase (EcoL) and ABTS into the base ink, respectively. 50 mM Na-succinate buffer (pH 4.5) was used to adjust the thickness of the ink. Compositions of the anode and cathode inks are listed in Table 2.

Classic Bar cardboard was selected as the printing substrate because it is rather thin (175 g m^{-2}) and therefore runs relatively well in the small printing machine. It also has a PE coating (15 g m^{-2}) making it heat-sealable in the post-processing and therefore simplifying the final assembly of the power source. In addition, the PE coating is beneficial for the moisture retention of the cell.

Water-based inks might be challenging when they are printed on plastic (here the PE coating of the substrate) due to their low viscosity and high surface tension. At the same time, plastics have low surface energy. Hence, a separate carbon-based current collector ink layer, which also acted as a conductive surface, was printed and dried on the PE-coated side of the substrate. The solvent of this ink was diethylene glycol monobutyl ether (2-(2-butoxyethoxy)ethanol), thus the ink-plastic interaction was not problematical due to the low surface tension of the ink. Enzyme containing inks were printed on the dry current collector layers.

In practice, current collectors were printed first in a separate print run, and they were dried and sintered at 145 °C (manufacturer's recommendation) in order to achieve high conductivity. Next, anode and cathode layers

were printed separately on the current collectors. Optimized drying temperature for enzyme inks was determined in the laboratory experiments (see Chapter 3.3.1), and it was taken as the starting point in the trial. The dryness of the printed layers was regularly checked during the trial. The drying temperature of the enzyme inks was first 65 °C, and this was found to be sufficient for the cathode ink. For the anode ink, 65 °C was too low and therefore the drying temperature was stepwise increased until a sufficient surface dryness was reached. The anode layer was eventually dried at 72 °C.

2.4 Electrochemical measurements

2.4.1 *Laboratory-scale fabricated enzymatic power sources*

The printed electrodes were cut from the substrate and assembled between graphite current collector plates. The printed layers were facing towards the graphite plates. The electrodes were separated by a double dialysis membrane, as described in previous publications [4, 6]. A palladium/hydrogen (Pd/H_2) reference electrode was placed into the membrane, between the anode and cathode, in chronopotentiometric measurement. The cells were activated with 50 mg of D-glucose on the anode and 200 µl of 50 mM Na-succinate buffer solution (pH 4.5) on each electrode; hence, the total amount of electrolyte was 400 µl.

The performance of the cells was measured using a potentiostat/galvanostat (VMP BioLogic). The cells were run in open circuit state for 90 min, after which either chronopotentiometric (constant current of 15 µA, three electrode connection) or power curve (5 µA current increase every 60 s from 0 µA until short-circuit current, two electrode connection) measurement was performed. The energy density of the cells was calculated from the chronopotentiometry by integrating the power over time, until the potential of the cell was under 200 mV. Two repetitions were measured in each measurement.

2.4.2 *Pilot-scale fabricated enzymatic power sources*

The printed electrodes were cut from the cardboard rolls for measurements. The activation of the cell was done by moisturizing the anode with a 200 µl buffer (50 mM Na-succinate pH 4.5) and 50 mg D-glucose, after which a piece of a separator was set on top of it. Both dialysis membrane and capacitor paper were tested as separator material. The cathode was moisturized with 100 µl buffer and put on top of the anode-separator assembly in such a way that the printed sides of the electrodes were facing each other (see Fig. 2). Next, each side of the rectangular cardboard cells

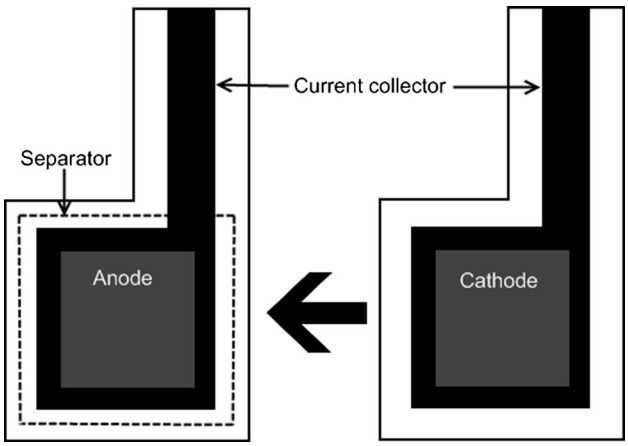

Fig. 2 Illustration of cell assembly using pilot-scale fabricated enzymatic electrodes

was heat-sealed by using a vacuum sealer (AVN AmeriVacS, setting: heat 6, cool 6).

The performance of the cells was measured using a potentiostat/galvanostat (VMP BioLogic). The cells were run at in open circuit state for 90 min, after which either chronopotentiometric (constant current of 10 µA, two electrode connection) or power curve (25 mV potential step every 600 s from 300 to 0 mV, two electrode connection) measurement was performed. Energy density of the cells was calculated from the chronopotentiometry by integrating the power over time until the potential of the cell was under 200 mV. At least three repetitions were measured in each measurement.

2.5 Enzyme activity measurements of printed electrodes

The detection of enzymatic activity of the printed electrodes was based on the oxygen consumption in the enzymatic reactions (see Eqs. 1 and 2 for the reactions for laccase and GOx, respectively) and measured using a fibre-optic oxygen meter (OXY-10 PreSens) for dissolved oxygen in solution. The principle of the sensor operation is based on the quenching of luminescence caused by collision between molecular oxygen and luminescent dye molecules in the excited state. As the numerical quantity value of enzyme activity is dependent on the experimental conditions, the values obtained here were only for relative comparison of different electrodes.

$$O_2 + ABTS \xrightarrow{laccase} H_2O + ABTS^+ \quad (1)$$

$$Glucose + O_2 \xrightarrow{GOx} gluconolactone + H_2O_2 \quad (2)$$

Air-saturated 50 mM Na-succinate buffer solution (pH 4.5) with 5 mM mediator (ABTS for laccase and FeMeOH for GOx) was used as the measurement solution. A sample

Table 3 Ink formula for drying experiments

Mass (g)	Ink component
32	PEO (5 w % in H_2O)
30	Graphite (G1.2)
0-15	50 mM Na-succinate buffer (pH 4.5)

(1 cm^2) of printed enzymatic electrode was placed into a vial (1.8 ml) with the measurement solution, and the amount of dissolved oxygen in the solution was measured versus time. The average slope of the oxygen-time curve was calculated and used to compare different samples with each other.

2.6 Drying experiments of inks

The running speed of the ROKO printing machine was 2 m/min (possible max 10 m/min) and it is consisted of an unwinding unit, screen-printing units, four air drying units and a winding unit (see Online Resource 2). The first goal of the drying experiments was to estimate the lowest dry matter content (DMC) of the dried ink that still allows R2R fabrication process. The second goal was to evaluate the correct drying temperature for the ROKO printing machine in order to achieve this DMC level. The third goal was to study how well the enzymatic layers retain their bio-electrochemical activity in planned drying conditions.

2.6.1 Drying experiments of graphite-based ink

The purpose of these experiments was to ascertain the DMC target and collect data for estimating the drying conditions for the ROKO printing machine. Laboratory experiments were implemented using a laboratory screen-printer (Miniprinter SMP-3) and laboratory oven (Memmert UE 500) with air circulation. The measurement of the moisture content of the ink layer was based on weighing the samples. The formula of the ink used is described in Table 3.

Printing substrate (polyimide sheet) was weighed after which an ink layer (36 cm^2) was printed on it. Printed substrates were weighed and dried in a laboratory oven. Printed and dried substrates were weighed, dried to 100 % DMC and re-weighed. The initial DMC, the amount of ink and the DMC of ink after first drying was calculated from these measurements. Surface dryness was determined by sensory evaluation.

Different ink amounts, two different drying temperatures (60 and 80 °C) and several drying times from 30 to 90 s were used. The initial DMC of the ink was also varied by adding buffer solution into the ink. Measured values were used in a preliminary drying model in order to get an assumption of the drying temperature for the pilot printing run.

2.6.2 Drying experiments of enzyme containing inks

The goal of these drying experiments was to ascertain the retention of enzymatic activity of dried enzyme ink layers in various drying conditions. Enzymatic electrodes were made as described in Chapter 2.3.1, expect that the amount of both anodic and cathodic mediators was 85 % lower, and the laccase was EcoL instead of ThL. Further, electrodes without the mediators were prepared. These samples were dried at four different temperatures: RT or 1 min at 50, 75 or 95 °C. After the drying the layers were stored either at RT or at 4 °C. The enzymatic activity of these samples was measured as described in Chapter 2.5 after 1, 14 and 28 days of storing. Two repetitive individual samples were used for each measurement.

2.7 Characterization of the morphology of R2R fabricated electrodes

The thickness of the printed electrodes was determined using a Dektak stylus profilometer. Roughness values were measured using a Wyko white light interferometer and the measured area was 0.91 mm × 1.20 mm. Three Au layers were sputtered on top of the samples prior to measuring. The number of samples was at least three and three measurement points were selected for each sample.

3 Results and discussion

The first trials were conducted in laboratory conditions in order to optimize the mediator selection for GOx, the ink composition for R2R processing and to retain the bio-electrochemical performance of the enzymes. Two different water-soluble biocompatible polymers were tested as binders for the enzymatic inks, and the printed layers were characterized electrochemically. Drying of these enzymatic inks was studied at different temperatures in order to ascertain how the drying affects the enzymatic activity of the printed electrodes. Finally, manufacturing of enzymatic electrodes was up-scaled to pilot-scale. Enzymatically active mass-produced printed anode and cathode layers were used to assemble fully enzymatic power sources. These cells were characterized electrochemically and their performance was compared to the power sources produced in the laboratory.

3.1 Mediator selection for glucose oxidase

The optimal mediator for the intended use should display a redox potential similar to that of the GOx enzyme (median reduction potential −0.008 V vs. NHE at pH 7 and 25 °C [13]). The most important property of the mediator is its

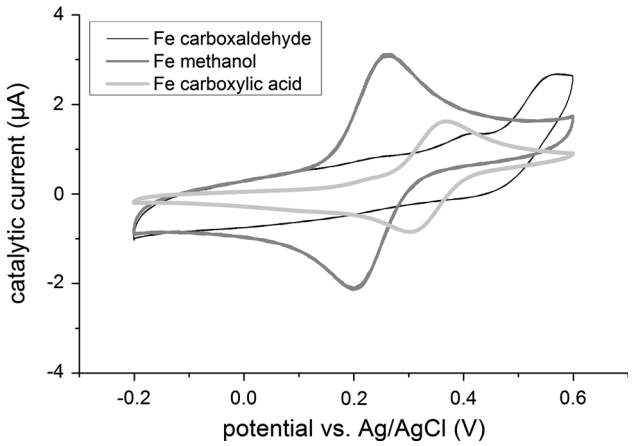

Fig. 3 Redox response on glassy carbon electrode of potential mediator alternatives for GOx

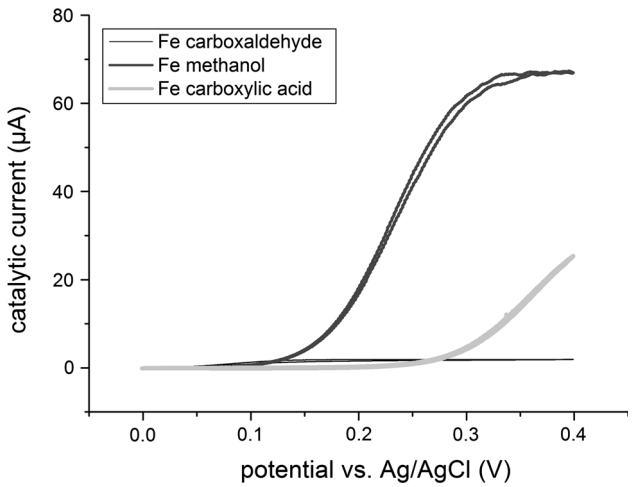

Fig. 4 Voltammetric response of different GOx/mediator systems during D-glucose oxidation

compatibility with the enzyme, ensuring as high a continuous glucose oxidation as possible at as low activation potential as possible. Its redox reaction should also be fully reversible in order to ensure a continuous operation without depletion of the active form of the mediator. Furthermore, it should be sufficiently stable (chemical/physical) with minimum deactivation as a function of storage time. The redox response of three potential mediators for GOx was tested. Redox responses of these tested mediators are shown in Fig. 3, and it can be seen, that FeMeOH meets the best the requirements mentioned above on redox response.

The voltammetric responses (see Fig. 4) revealed that the current output of glucose oxidation is also largest with FeMeOH, which is most probably due to compatible redox potential of the enzyme-mediator couple. Based on these tests FeMeOH was selected as the mediator for GOx electrodes.

3.2 Electrochemical characterization of cells using different binders

The viscosity of ink can be tailored with different polymer binders. Due to the use of enzymes as catalysts in our bio power sources, water was selected as the solvent for the inks. Water is a very environmentally friendly solvent and economic as well. Additionally, water is a very popular solvent with industry, particularly for biocatalytic procedures [14]. Graphite powder was selected as the main component of the ink in order to form a conductive electrode layer when the ink has been dried. Promising water-soluble binders for enzymatic inks are polyethylene oxide (PEO) and carboxymethyl cellulose (CMC). PEOs are polymers of ethylene oxide, which are represented by the formula $(O–CH_2–CH_2)_n$ in which n represents the average number of oxyethylene groups [15]. PEOs are non-ionic, water-soluble and highly hydrophilic. Therefore, they are characterized by their flocculent, thickening, sustained-release, lubrication, dispersing, and water-retention properties [16]. PEOs are widely used in different applications. In Li-ion battery technology they have been used as a host polymer in electrode layers [17, 18] and in medicine e.g. in injectable medicine systems [16] and hydrogels [19]. CMC is a linear polymeric derivative of cellulose. They consist of linked glucopyranose residues with varying levels of carboxymethyl (–CH2COO–) substitution. The carboxymethyl groups are responsible for the aqueous solubility of CMC relative to insoluble cellulose. CMC acts as a weak polyacid that dissociates to form carboxylate anionic functional groups [20]. Due to its safety and solubility in water, CMC is used in a wide range of applications. For example, in food industry CMC is used as viscosity modifier and thickener [21] and as a packaging material [22]; in battery technology it has been studied as a constituent in alternative anodes for Li-ion batteries [20, 23].

Due to these desirable characteristics of PEO and CMC, both polymers were selected for laboratory experiments as binders for enzyme-containing inks, and their effect on the electrochemical properties of the enzymatic electrodes was studied. Both power and current–potential curves (see Fig. 5) in potentiometric mode as well as a chronopotentiometry (see Fig. 6) were measured.

The open circuit potential (OCP), and maximum power, current and energy density of PEO binder based cells are 380 ± 8 mV, 1.4 ± 0.1 µW cm^{-2}, 8.35 ± 0.04 µA cm^{-2} and 5.5 ± 0.2 µWh cm^{-2}, respectively. The OCP, and maximum power, current and energy density of CMC binder based cells are 270 ± 1 mV, 0.51 ± 0.02 µW cm^{-2}, 4.45 ± 0.01 µA cm^{-2} and 3.3 ± 0.1 µWh cm^{-2}, respectively.

These results show that the cells that have PEO as the binder have 1.4 times higher OCP, 2.7 times higher maximum power density, 1.9 times higher maximum current

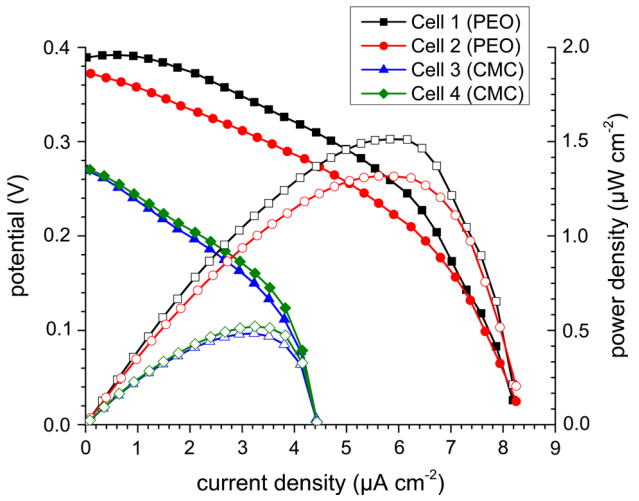

Fig. 5 Potential-current (*full symbols*) and power (*hollow symbols*) curves of cells constructed using laboratory-scale printed electrodes. The measurement was done by increasing current load by 5 μA every 60 s in two electrode connection. Two different polymers were tested as the binder of the enzymatic inks, and two repetitions were measured for each binder (Cell 1 and 2 with PEO, Cell 3 and 4 with CMC)

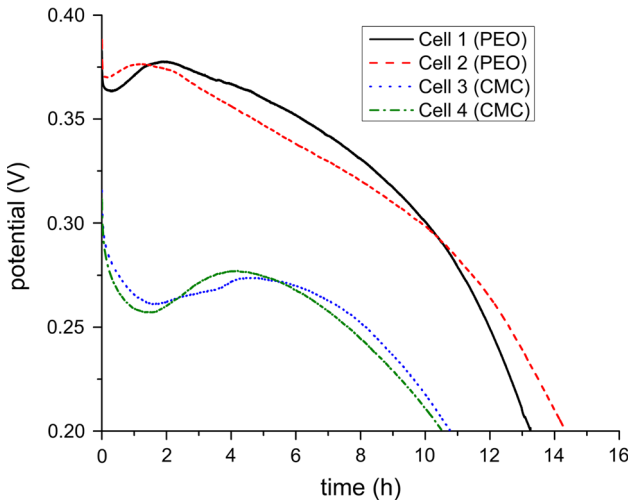

Fig. 6 Cell potential of GOx//ThL cells constructed using laboratory manufactured electrodes. The chronopotentiometric measurement (constant current of 1.2 μA cm^{-2}) was done using three electrode connection with Pd/H_2 reference electrode between the electrodes (see Online Resource 3 for separate electrode potentials). Two different polymers were tested as the binder of the enzymatic inks, and two repetitions were measured for each binder (Cell 1 and 2 with PEO, Cell 3 and 4 with CMC)

density and 1.7 times higher energy density. PEO works better as the binder for both GOx and laccase. The difference between PEO and CMC is significant especially with the laccase ink. The potential of the cathode is approximately 100 mV lower when CMC is used as the binder (around 300–350 mV for PEO and 200–250 mV for CMC, see Online Resource 3). Enzyme activity measurement shows that CMC deactivates the oxygen reduction reaction of laccase (see Online Resource 3). This is most probably due to the anionic property of CMC, which is commonly used in ion exchange chromatography for purification of enzymes [24, 25]. The theoretical isoelectric point of ThL is 4.81, thus the charge of the laccase in our setup (pH 4.5) is positive. The pK_a of CMC in our setup (50 mM Na-succinate) is close to 4 [26, 27], hence it acts as a resin, retaining positive molecules based on columbic interactions. Consequently, positively charged laccase enzymes are trapped into the structure of CMC forming insoluble complexes. Due to the deactivation of the laccase by CMC, PEO was selected as the binder for further experiments.

In the previous work by Jenkins et al. [6], fully enzymatic printed power source using GOx/TMPD as the anode and ThL/ABTS as the cathode were produced. With TMPD as the anodic mediator, they received a maximum power density of 0.28 μW cm^{-2} and an energy density of 0.12 μWh cm^{-2}. Hence, by using FeMeOH as the anodic mediator we were able to improve the power density fivefold and the energy density 28 fold.

Zhang et al. [28] have presented a mediator-less and compartment-less paper-based biofuel cell (GOx//bilirubin oxidase, BOD) chip of a size of 1.5 cm × 1.5 cm. The maximum power density was 13.5 μW cm^{-2}. The chip was operational for 45 min with 30 μl of electrolyte. Shitanda et al. [29] have demonstrated a paper-based biofuel cell (GOx//BOD) fabricated using screen-printing. The area of the electrodes was 0.5 cm × 0.5 cm. They achieved a maximum power density of 0.12 mW cm^{-2}. Although both of these studies showed higher power densities compared to our printed biofuel cells, there were significant differences in the electrodes: The specific enzyme loading was at least 100 times higher than in our case. Additionally, both Zhang et al. and Shitanda et al. used either carbon nanotubes (CNT) or carbon black respectively in order to increase the 3D-area of the electrodes. In our case, no mesoporous carbon particles were used. Hence, our biofuel cells function relatively efficiently compared to the other paper-based printed biofuel cells. However, in future work the performance of our paper-based biofuel cells should be tested with addition of CNTs, graphene or carbon black into the enzymatic inks.

3.3 Drying experiments on inks

3.3.1 Drying experiments on the graphite base ink

The DMC that corresponds to sufficient surface dryness is roughly 88 % according to drying experiments in the laboratory. Determinations were made by drying the printed samples at different temperatures and with various inks. It

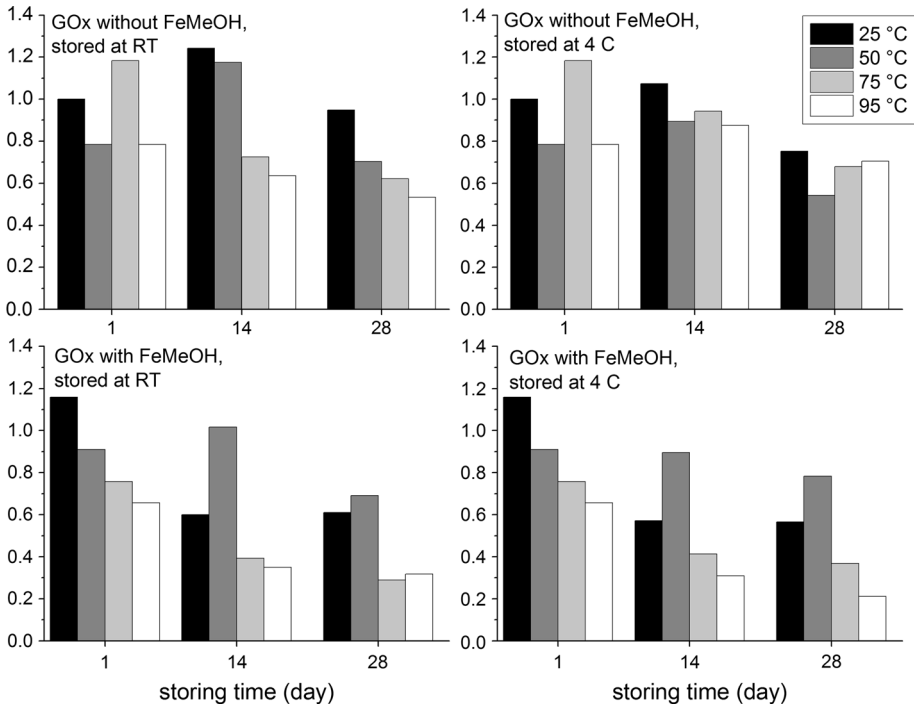

Fig. 7 Proportional (compared to electrodes without mediator, dried and stored at RT) enzyme activity of printed GOx electrodes; the electrodes were dried at RT, 50, 75 and 95 °C, and stored both at RT and 4 °C. Two repetitions were measured at each measurement point

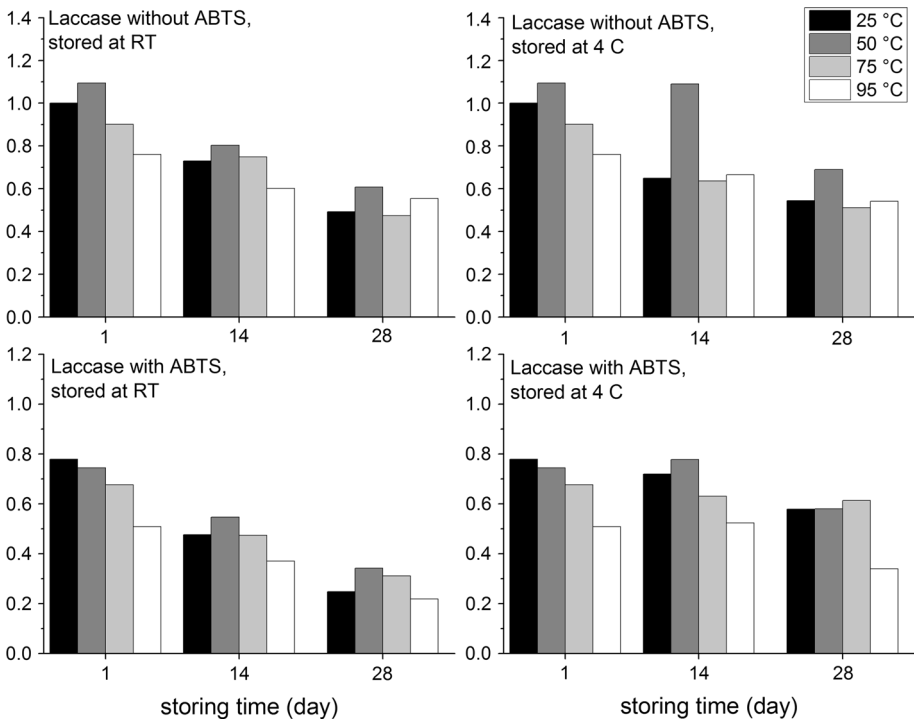

Fig. 8 Proportional (compared to electrodes without mediator, dried and stored at RT) enzyme activity of printed laccase (EcoL) electrodes, the electrodes were dried at RT, 50, 75 and 95 °C, and stored both at RT and at 4 °C. Two repetitions were measured at each measurement point

was calculated and modelled from the data that 65 °C drying temperature should be sufficient for the ROKO printing line.

3.3.2 Drying experiments on the enzyme containing inks

The curves of relative O_2 consumption of printed enzymatic electrodes are presented in Figs. 7, 8 for GOx and laccase (EcoL), respectively. The first observation is that both the storage time and drying temperature affect the catalytic activity of both enzymes.

The GOx electrodes without FeMeOH that are dried and stored at RT (GOx reference electrodes) retain 95 % of their initial activity after 28 days. When GOx electrodes are dried at 95 °C, their initial activity is 78 % compared to the GOx reference electrodes, and 53 % after 28 days.

Storing the GOx electrodes at 4 °C shows some effect: The electrodes that are dried at RT and 95 °C show 75 and 71 % of activity after 28 days, respectively, compared to the GOx reference electrodes. It seems that, especially in the case of a higher drying temperature, storing GOx electrodes at 4 °C has a beneficial effect on stability.

The initial activity of the GOx electrodes with FeMeOH that are dried at RT is 114 % compared to the GOx reference electrodes. When the same comparison is made after 28 days with the electrodes that are stored at RT, the activity is 61 % (vs. 95 % without FeMeOH). For electrodes that are stored at 4 °C, the activity is 57 % (vs. 75 % without FeMeOH) compared to the GOx reference electrodes. This data indicates that the addition of FeMeOH to the GOx ink deactivates the enzyme during storage. The reason for this could be formation of an irreversible enzyme-mediator complex during storage which prevents the glucose oxidation with oxygen as the electron acceptor in the activity measurement. It must be emphasised that this phenomenon is not fully understood.

Laccase (EcoL) electrodes without ABTS that are dried and stored at RT (laccase reference electrodes) retain 49 % of their initial activity after 28 days. When these electrodes are dried at 95 °C, their initial activity is 76 % compared to the laccase reference electrodes, and 55 % after 28 days. Storing the electrodes at 4 °C does not show much effect: After 28 days, both electrodes dried either at RT or at 95 °C show 54 % compared to the laccase reference electrodes.

The initial activity of the laccase electrodes with ABTS that are dried at RT is 78 % compared to the laccase reference electrodes. When the same comparison is made after 28 days to the electrodes that are stored at RT, the activity is 25 % (vs. 49 % without ABTS). For electrodes that are stored at 4 °C, the activity is 58 % (vs. 54 % without ABTS) compared to the laccase reference electrodes. As in the case of GOx electrodes, adding mediator into the laccase ink deactivates the electrodes during storage, although storing the electrodes at 4 °C seems to retain the enzymatic activity well when compared to the reference case. The reason for reduced enzymatic performance of laccase electrodes in the presence of ABTS can be related to instability of the mediator. This phenomenon is not fully understood and the authors suggest further studies in order to gain better understanding of the stability of printed enzymatic electrode systems.

It has been noticed before by Smolander et al. [3] that the activity of ThL is maintained in printed dry layers for several weeks, even months. For example, carbon nanotube-based ThL/ABTS ink retained at 100 % after 2 weeks of storage, and 82 % after 6 weeks of storage at 22 °C. It the same article, heat treatment of ThL was tested. One minute of heat treatment at 50 °C resulted in 65 % residual activity and at 90 °C in 27 % residual activity. In our work, the heat treatment did not have much effect on the enzymatic activity of the laccase. The reason might be the use of different laccases: EcoL is a commercial laccase used in pre-treatment of textiles, which has been most probably tailored to be robust.

In some cases, the increased drying temperature (50 °C) had a positive effect on the enzyme activity. The improvement in enzymatic activity compared to the electrodes dried at RT might be due to the formation of a dry ink layer around the enzyme that protects the enzyme against conformational changes. However, when the drying temperature increases, it causes deactivation of the enzyme. Another explanation might be morphological changes of the ink layer; different drying temperatures will most probably lead to various micro- and mesoporous structures. Different porosities have various diffusion properties (e.g. for glucose, oxygen, mediator), which might be seen as differences in enzymatic activity. On the other hand, Farnet et al. [30] have found that pre-incubation of enzymes at 40 and 50 °C increased laccase activity, and this effect might have been visible in the case of both GOx and laccase.

The main conclusion is that both GOx and laccase containing enzymatic inks retain their enzymatic activity even though they are dried for 1 min at 95 °C. This was requisite information for the later pilot printing trial because the preliminary estimated drying condition was 65 °C (see Chapter 3.3.1).

3.4 R2R production of enzymatic electrodes

Pilot-scale printing trials focused on two primary subjects; (1) drying of anode and cathode ink layers and (2) runnability issues. Drying is a critical step of manufacturing printable enzymatic power sources. Printed surfaces must be dry enough so that they can be handled in post-processing steps but they cannot be excessively dried to avoid loss of enzyme activity. Overall, the R2R production of enzymatic electrodes was achieved successfully (a picture of a roll of enzymatic electrodes can be found in Online Resource 4).

3.5 Morphology of R2R printed layers

All printed layers are rough, due to the small mesh count of the printing screens used. The anode and cathode layers are approximately 26 and 37 % thicker than the current collector layers, respectively. In addition, the roughness values of anode and cathode layers were approximately 3.4 and 5.5 times higher than the current collector layers, respectively. The average thickness and roughness values are listed in Table 4, and more data can be seen in Online Resource 5.

Table 4 Average thickness and roughness of printed layers

	Thickness (μm)	Ra[a] (μm)	Rq[b] (μm)
Current collector	82 ± 11	2.6 ± 0.3	3.3 ± 0.3
Anode	103 ± 22	9 ± 1	11 ± 3
Cathode	112 ± 24	14 ± 2	19 ± 4

[a] Arithmetic mean

[b] Quadratic mean

The sheet resistance of the current collector ink was expected to be 13–20 Ω with a 25 μm thick layer when the ink is dried at 130–150 °C for 45 min (values are given by the manufacturer). In reality, a sheet resistance of 16–17 kΩ was obtained for the current collector layers in the conditions used. The sheet resistance of anode and cathode layers was approximately 4 kΩ.

The reason for such a high sheet resistance of the current collector layer is the different drying conditions compared to the manufacturer's recommendations. The drying temperature for current collector layers was 145 °C but the drying time was only 81 s. For this reason, the layers did not sinter adequately, leading to low conductivity values.

3.6 Electrochemical characterization of the cells assembled with R2R-produced electrodes

During assembly of the cells, it is observed that the anode ink cracks very easily, which makes it difficult to assemble the cells. The anode ink has an increased water-solubility, which most probably reduces the functionality and operational lifetime of the cell, because the enzyme is not immobilized into the ink structure and the conductive layer will be lost. The cathode ink layer is more rigid than the anode ink layer.

The power and current–potential curve from amperometric measurement using different separator materials are shown in Fig. 9. In addition, results from chronopotentiometric measurement are shown in Fig. 10. Two different separator materials were tested: capacitor paper and dialysis membrane. The maximum power densities are 0.16 ± 0.02 and 0.40 ± 0.03 μW cm^{-2} for capacitor paper and dialysis membrane, respectively. The maximum current densities are 1.97 ± 0.03 and 2.78 ± 0.04 μA cm^{-2} for capacitor paper and dialysis membrane, respectively. The calculated average energy density of the cells with dialysis membrane separator is 0.6 ± 0.1 μWh cm^{-2}.

The results show that the cells having dialysis membrane as the separator perform better than the cells having capacitor paper as the separator. This might be due to different physical properties of the separators. The capacitor paper is a porous material which probably does not separate the anode and cathode materials well enough.

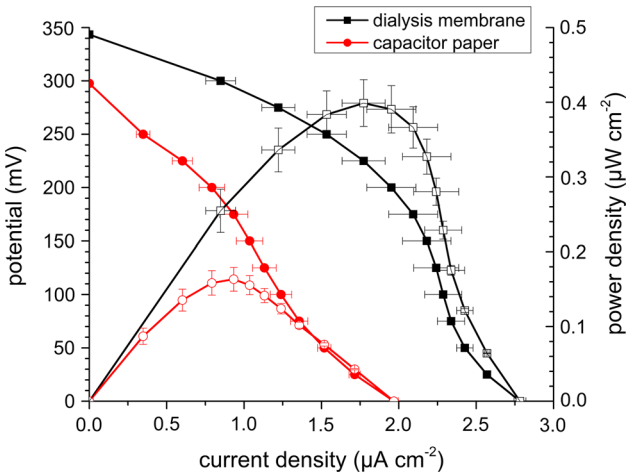

Fig. 9 Potential-current (*full symbols*) and power (*hollow symbols*) curves of pilot printed GOx//EcoL cells with two different separator materials. The measurement was done by 25 mV potential steps every 10 min, from 300 to 0 mV. Three repetitions were measured

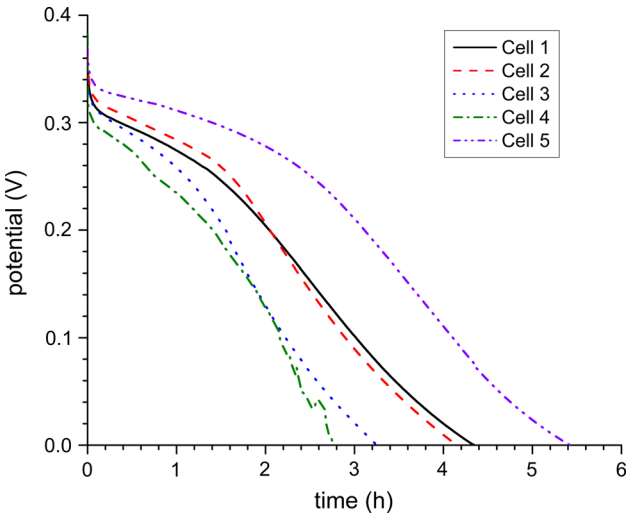

Fig. 10 Curve of chronopotentiometric measurement (constant current of 1.1 μA cm^{-2}) of GOx//laccase cells constructed from pilot-scale printed electrodes. Dialysis membrane was used as the separator. Five repetitions were measured

High cross-over of the mediators and enzymes might reduce the performance of the cell. Dialysis membrane, on the other hand, works better than capacitor paper most probably due to the smaller pore size of the material than that of the capacitor paper. In addition, it is noticed that the dialysis membrane absorbs water, which causes swelling. Consequently, it can work as a lamination component between the anode and cathode layers. For this reason, dialysis membrane was selected as the separator for further experiments.

Both the maximum power and energy density are only 11 % of the best laboratory manufactured cells. There are several reasons for the reduced performance. First of all, the

construction of the cells was different. The laboratory test cells were measured inside graphite current collectors. In the case of pilot printed electrodes a separate ink layer, under the anode and cathode layers, acted as the current collector. The high sheet resistivity of the current collector ink layer (see Chapter 3.5) leads to high ohmic losses in the cell.

Secondly, the amount of mediators was 75–76 % lower in the inks used in the pilot run. This is expected to be seen as reduced electrochemical performance, which is dependent on the mediator amount. In addition, increasing drying temperature reduces the enzymatic activity of the electrodes, as described in Chapter 3.3.2. We have noticed before that the anode electrode is the limiting electrode in our cells [31], and drying the anode at 72 °C for 81 s reduces the activity by approximately 40 %. When these two factors, that is to say reduced mediator amount and increased drying temperature, are calculated together, the performance can be estimated to be 15 % of the laboratory-scale cells. Adding the ohmic losses from the printed current collectors and possible delamination effects of the anode and cathode layers reduces the performance further. Thus the finding that the pilot manufactured cells showed 11 % of the best laboratory manufactured cells is reasonable. Increasing the amount of mediators, curing the printed current collectors at sufficient conditions (e.g. IR heating for 2 min at 180 °C as suggested by the manufacturer), as well as laminating the layers better together should be tested in order to increase the performance of the pilot manufactured cells. Some of these suggestions will be studied in the future work conducted by the authors.

4 Conclusions

Production of printable enzymatic power sources was scaled up from laboratory-scale to R2R pilot production scale. We noticed that PEO works well as a binder for enzymatic inks, but CMC seems to deactivate the laccase enzyme. The reason is most probably due to the anionic characteristics of CMC. With PEO as the binder and Fe-MeOH as the new anode mediator, we were able to improve the power density fivefold and energy density 28 fold compared to our previously published values of printed GOx//laccase cells.

Drying inks in the R2R process is an important step, hence different drying temperatures for the enzymatic inks were studied. We noticed that both GOx and laccase containing enzymatic inks retained their enzymatic activity even though they were dried as high as at 95 °C. The residual enzymatic activities were 57 % for GOx and 65 % for laccase, compared to electrodes dried at RT. It was noticed that the samples that did not contain any mediator in the ink had better stability than the samples with mediators.

R2R manufacturing of enzymatic electrodes was piloted using rotary screen-printing in VTT's modular ROKO printing machine with a printing speed of 2 m/min. Rolls of enzymatically active printed anode and cathode layers were produced, and these were used to assemble fully R2R-printed enzymatic power sources. The electrochemical performance of the pilot cells was approximately 11 % of the cells produced in laboratory. The reason for the reduced performance is most probably due to the lower mediator amount and higher drying temperature than that of the laboratory cells. All things considered, these trials showed that printed enzymatic active layers can be successfully fabricated with the ROKO printing machine in R2R process. Hence, GOx//laccase cells could be produced on a large scale.

Acknowledgments Tiina Maaninen, Pekka Ontero, Mikko Hietala and Arto Ranta-Panula (VTT) are thanked for their assistance in the R2R manufacturing of enzymatic electrodes. In addition, Asta Pesonen (VTT) and Prof. Kyösti Kontturi and Dr. Tanja Kallio (Aalto University) are gratefully thanked for technical and academic assistance. The research was part of the Printed Enzymatic Power Source with embedded capacitor on next generation devices –project (PEP-Second) supported by TEKES, the Finnish Funding Agency for Technology and Innovation. Co-funding by companies is gratefully acknowledged.

References

1. Das R, Harrop P (2011) Printed, organic & flexible electronics forecasts, players & opportunities 2011–2021. Printed and potentially printed—the complete picture. IDTechEx
2. Chansin G (2013) Printed and flexible sensors 2014–2024: technologies, players, forecasts. Established & emerging markets—the complete picture: biosensors, temperature sensors, gas sensors, capacitive sensors, piezoresistive sensors, piezoelectric sensors, photodetectors. IDTechEx
3. Smolander M, Boer H, Valkiainen M, Roozeman R, Bergelin M, Eriksson J-E, Zhang X-C, Koivula A, Viikari L (2008) Development of a printable laccase-based biocathode for fuel cell applications. Enzyme Microb Technol 43:93–102
4. Tuurala S, Smolander M, Uotila J, Kaukoniemi O-V, Boer H, Valkiainen M, Vaari A, Koivula A, Jenkins P (2010) Performance of a printable enzymatic fuel cell: study on mediated ThL laccase cathode. ECS Trans 25:1–10
5. Tuurala S, Lau C, Atanassov P, Smolander M, Minteer SD (2012) Characterization and stability study of immobilized PQQ-dependent aldose dehydrogenase bioanodes. Electroanalysis 24:229–238
6. Jenkins P, Tuurala S, Vaari A, Valkiainen M, Smolander M, Leech D (2012) A comparison of glucose oxidase and aldose dehydrogenase as mediated anodes in printed glucose/oxygen enzymatic fuel cells using ABTS/laccase cathodes. Bioelectrochemistry 87:172–177
7. Jenkins P, Tuurala S, Vaari A, Valkiainen M, Smolander M, Leech D (2012) A mediated glucose/oxygen enzymatic fuel cell based on printed carbon inks containing aldose dehydrogenase and laccase as anode and cathode. Enzyme Microb Technol 50:181–187

8. Kipphan H (2001) Handbook of print media: technologies and production methods. Springer, Berlin
9. Gilleo K (1989) Rheology and surface chemistry for screen printing. Screen Print Magaz 128–132
10. Frasconi M, Favero G, Boer H, Koivula A, Mazzei F (2010) Kinetic and biochemical properties of high and low redox potential laccases from fungal and plant origin. Biochim Biophys Acta 1804:899–908
11. Rittstieg K, Suurnakki A, Suortti T, Kruus K, Guebitz G, Buchert J (2002) Investigations on the laccase-catalyzed polymerization of lignin model compounds using size-exclusion HPLC. Enzyme Microb Technol 31:403–410
12. Wang X, Sjöberg-Eerola P, Eriksson J-E, Bobacka J, Bergelin M (2010) The effect of counter ions and substrate material on the growth and morphology of poly(3,4-ethylenedioxythiophene) films: towards the application of enzyme electrode construction in biofuel cell. Synth Met 160:1373–1381
13. Duke FR, Kust RN, King LA (1969) The standard potential of glucose oxidase. J Electrochem Soc 116(1):32–34
14. Kerton FM, Marriot R (2013) Alternative solvents for green chemistry. The Royal Society of Chemistry, United Kingdom
15. Dhawan S, Varma M, Sinha VR (2005) High molecular weight poly(ethylene oxide)-based drug delivery systems—Part I: hydrogels and Hydrophilic Matrix Systems. Pharm Technol 29:72–80
16. Dhawan S, Dhawan K, Varma M, Sinha VR (2005) Applications of Poly(ethylene oxide) in drug delivery systems—Part II. Pharm Technol 29:82–96
17. Ramesh S, Ramesh K, Arof AK (2013) Fumed silica-doped poly(vinyl chloride)-poly(ethylene oxide) (PVC/PEO)-based polymer electrolyte for lithium ion battery. Int J Electrochem Sci 8:8348–8355
18. Hueso KB, Armand M, Rojo T (2013) High temperature sodium batteries: status, challenges and future trends. Energy Environ Sci 6:734–749
19. Hoffman AS (2012) Hydrogels for biomedical applications. Adv Drug Deliv Rev 64:18–23
20. Lee J-H, Paik U, Hackley VA, Choi Y-M (2005) Effect of carboxymethyl cellulose on aqueous processing of natural graphite negative electrodes and their electrochemical performance for lithium batteries. J Electrochem Soc 152:A1763–A1769
21. Diftis N, Kiosseoglou V (2003) Improvement of emulsifying properties of soybean protein isolate by conjugation with carboxymethyl cellulose. FoodChemistry 81:1–6
22. Tharanathan RN (2003) Biodegradable films and composite coatings: past, present and future. Trends Food Sci Technol 14:71–78
23. Li J, Lewis RB, Dahn JR (2007) Sodium carboxymethyl cellulose—a potential binder for Si negative electrodes for Li-ion batteries. Electrochem Solid State Lett 10:A17–A20
24. Madhavi V, Lele SS (2009) Laccase: properties and applications. BioResources 4(4):1694–1717
25. Janson J-C (2011) Protein purification—principles, high resolution methods, and applications. Wiley, New Jersey
26. Peterson EA, Sober HA (1956) Chromatography of Proteins. I. cellulose ion-exchange adsorbents. J Am Chem Soc 78:751–755
27. Zhivkov AM (2013) Electric properties of carboxymethyl cellulose. In: van de Ven T, Godbout L (ed) Cellulose—fundamental aspects. doi:10.5772/56935
28. Zhang L, Zhou M, Wen D, Bai L, Lou B, Dong S (2012) Small-size biofuel cell on paper. Biosens Bioelectron 35:155–159
29. Shitanda I, Kato S, Hoshi Y, Itagaki M, Tsujimura S (2013) Flexible and high-performance paper-based biofuel cells using printed porous carbon electrodes. Chem Commun 49:11110–11112
30. Farnet AM, Criquet S, Tagger S, Gil G, Le Petit J (2000) Purification, partial characterization, and reactivity with aromatic compounds of two laccases from *Marasmius quercophilus* strain 17. Can J Microbiol 46(3):189–194
31. Tuurala S, Smolander MH, Valkiainen M, Vaari A, Ojala V-M, Bergelin M (2010) Measurement setup to study the electrode potentials separately in a printed biofuel cell. Abstract No. 420, 217th ECS Meeting, The Electrochemical Society